Anopheles egg concentration and bleaching V 2

Background:

Many laboratories (including the MR4) find that bleaching eggs is a helpful routine procedure to minimize the growth of microsporidia [1]. Mortality due to these pathogens is sometimes observed only after several generations of culture without surface sterilization. The following method describes a method to surface sterilize eggs and also allows for concentrating eggs on a filter paper disk for shipment or quantification.

Equipment:

1. Filtration device (A Buchner funnel is an option, but take care to clean carefully if used for several stocks since eggs will leak around the edge of the paper). This one is made from a Nalgene 500 ml disposable filter by breaking off the upper chamber and removing the membrane. It can be reused indefinitely.
2. Filter paper. A heavy type that does not disintegrate quickly in water is recommended (e.g. Whatman, Grade 3 Qualitative Filter Paper, Catalog No. 1003 090)
3. 1% household bleach in a wash bottle
4. Clean larval culture water in a wash bottle
5. Vacuum source equipped with trap

Prodedure:

1) Remove adults from surface by filtering through screen or with forceps.
2) Center filter paper on platform with vacuum applied.
3) Slowly pour water/eggs onto the center of the disk so that eggs do not spill outside of the depressed region.
4) Wash the remaining eggs from the dish onto the center of the disk using bleach solution.
5) Remove vacuum.
6) Soak for up to 1 minute by adding bleach solution. Remove bleach by applying vacuum.
7) Add water until depression is full.
8) Apply vacuum.
9) Wash with water two times.
10) Apply vacuum. Eggs may be stored in a humid container on filter paper overnight before hatching.

1. E Alger, AH Undeen: The control of a microsporidian, Nosema sp., in an anopheline colony by an egg-rinsing technique. *J Invertebr Pathol* 1970, **15**:321-7.
2. V. Robert, T. Tchuinkam, P. Carnevale: Successful Eradication of a Microsporidian, Nosema sp., in a Mosquito Colony. *Ann. Soc. belge Med. trop.* 1993, **73**:71-72.